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Tokyo Medical and Dental University

Yokohama City University Graduate School of Medicine

Takeda-CiRA joint program（T-CiRA）

This press release is a translation of the Japanese press release issued on September 8, 2020. In the event of the provisions resulting in a conflict between the original press release and this release, the original will supersede

「 Polygenic architecture informs potential vulnerability to drug-induced liver injury 」
～Predict the susceptibility of disease from the genome～

【Highlights】

- The polygenic risk score (PRS)※1, by aggregating effects of over 20,000 SNPs※2, predicted the susceptibility to DILI (drug-induced liver injury)※3.
- Pathway analysis highlighted processes previously implicated in DILI, including unfolded protein responses and oxidative stress using human iPS cell-derived liver organoids※4 and primary hepatocytes.
- Individuals with high PRS have been shown to be clinically more likely to develop DILI by using clinical trial data.
- This research potentially will contribute to prospective designs of safer, more efficient, and robust pre-clinical/clinical study in drug development process.
- The proposed “polygenicity-in-a-dish” strategy, combining human cells and polygenic risk score, will be expected to development of personalized medicine and preventive medicine for various diseases.

The research group of Professor Takanori Takebe of Tokyo Institute of Medical and Dental Research (affiliation at the start of research, Yokohama City University) conducted this research in collaboration with Takeda Pharmaceutical Company Limited and Kyoto University iPS Cell Research Institute (CiRA). As part of the T-CiRA Joint Program ※5, it was shown that PRS (polygenic risk score) calculated from

individual genomic information can predict susceptibility to DILI (drug-induced liver injury) using human iPS cell-derived liver organoids, human hepatocytes and clinical trial data.

In the future, the achievement of this research will lead to the development of preclinical / clinical studies that can accurately predict DILI, which is one of the major termination factors for drug development. Furthermore, a new “polygenicity-in-a-dish” strategy, combining human cells and polygenic risk score, will be expected to development of personalized medicine and preventive medicine for various diseases, not only DILI.

The results of this research will be published online in the international science journal Nature Medicine, at 0:00 am, GMT on 7, September 2020 EST.

【Introduction】

Drug-induced liver injury (DILI) is a rare side effect that develops 1 in 10,000 to 100,000 individuals, but it is one of the leading causes of termination in drug development programs and drug withdrawals from the market. It was reported that drug development failure in phase lll clinical trials results in the loss of $1800M (1800 oku-yen) for 12 years. Thus, it is necessary to more accurately predict the toxicity of drug candidates in the preclinical studies. Recently, genome-wide association studies (GWAS) ※8 such as have been conducted by the international Drug-Induced Liver Injury Consortium (iDILIC) ※6 and the Drug-Induced Liver Injury Network (DILIN) ※7 identified several significant variants conserved across DILI due to multiple different drugs. However, it has been suggested that the prediction of DILI onset by specific genetic polymorphisms is relatively limited.

Since each variant has modest predictive impact, we herein revisited GWAS※8 findings to determine whether the polygenic score, which sums up effects of numerous variants, informs potential DILI susceptibility in humans. The goal of this study was to develop a polygenic risk scores (PRS) from GWAS data obtained in the DILI networks and to validate their significance in GWAS data obtained from an independent clinical trial of a hepatotoxic drug, as well as multiple donor-derived organoids and primary hepatocytes treated with a variety of hepatotoxic medications. We also tried to identify susceptibility-associated mechanisms.

【Results】

1. Polygenic risk scores stratify DILI risk susceptibility in multiple drugs assays of cultured human hepatocytes.

Based on summary statistics of a GWAS provided from the iDILIC/DILIN collaboration which studied 862 case individuals of DILI due to more than 150 drugs, the genetic polymorphism used for PRS and the weight used for risk calculation were decided.

We investigated whether the PRS could stratify DILI vulnerability using both human hepatocyte model

and human liver bud organoids (HLOs) derived from iPSC. Whole-genome genotypes of total 26 donors of primary human hepatocytes (n=21) and iPSC-derived liver organoids (n=5) were determined by SNP array to calculate their PRS. We experimentally evaluated cell viability of scored 26 donors under treatment of 12 drugs which reported to cause DILI. The result showed that the negative correlation patterns were plotted between the PRS and cell viability for each drug treatment. In other words, donors with high PRS tended to have lower survival rates. Taken together, these data show that the PRS-based stratification approach by the 26 donors derived hepatocyte cell-based assay was more widely applicable for multiple drug evaluation.

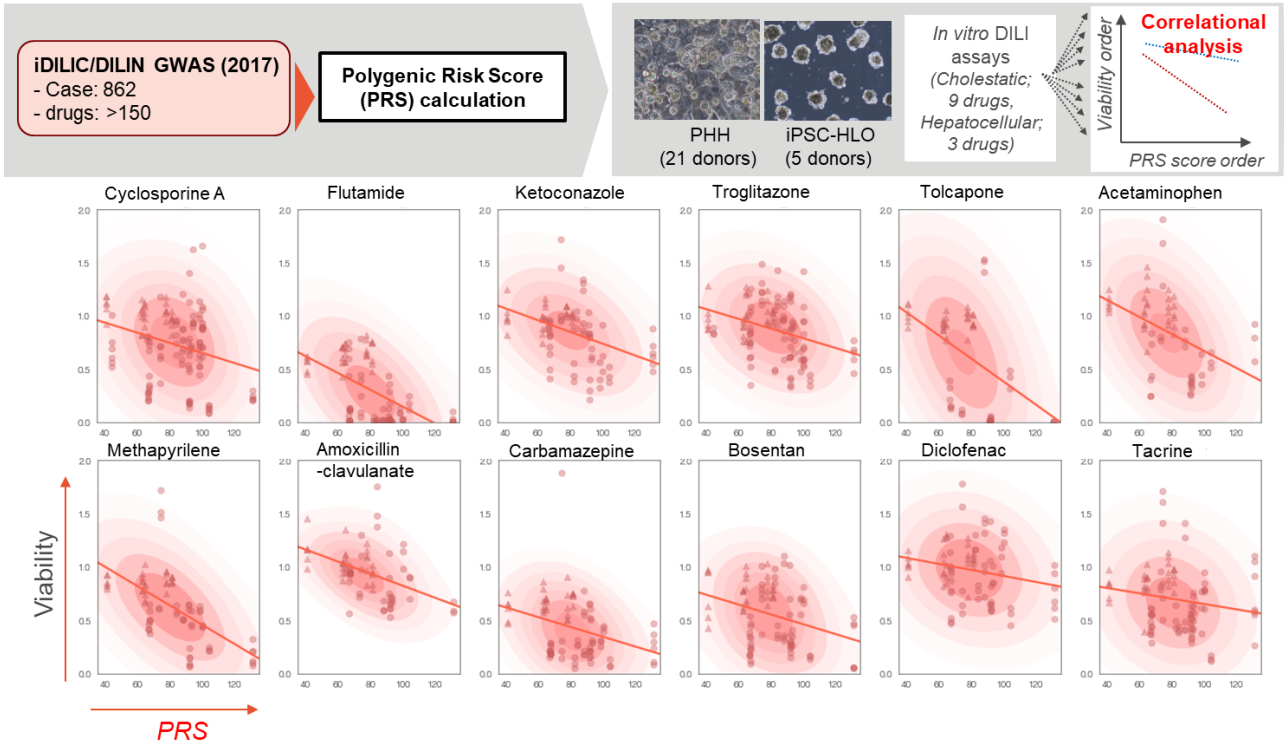


Figure1. Viability comparison of multi-donor iPSC-HLO models (triangle) and primary human hepatocytes (circle) under multiple DILI drug treatment.

2. Identification of DILI susceptible humans by polygenic risk score.

Next, we investigated whether the PRS could predict DILI in the clinical setting. In this study, we examined whether PRS could separate the control group from the DILI-affected group, using the clinical trial data that caused DILI due to Fasiglifam, Flucloxacillin, and Amoxicillin-Clavulanate. As a result, the PRS showed a significant difference between the control group and the DILI-onset group. We found that PRS potentially predicted susceptibility to DILI of 39 Fasiglifam, 167 flucloxacillin or 207 amoxicillin-clavulanate patients with DILI phenotype. Thus, clinical trial data also suggested that PRS could predict DILI susceptibility.

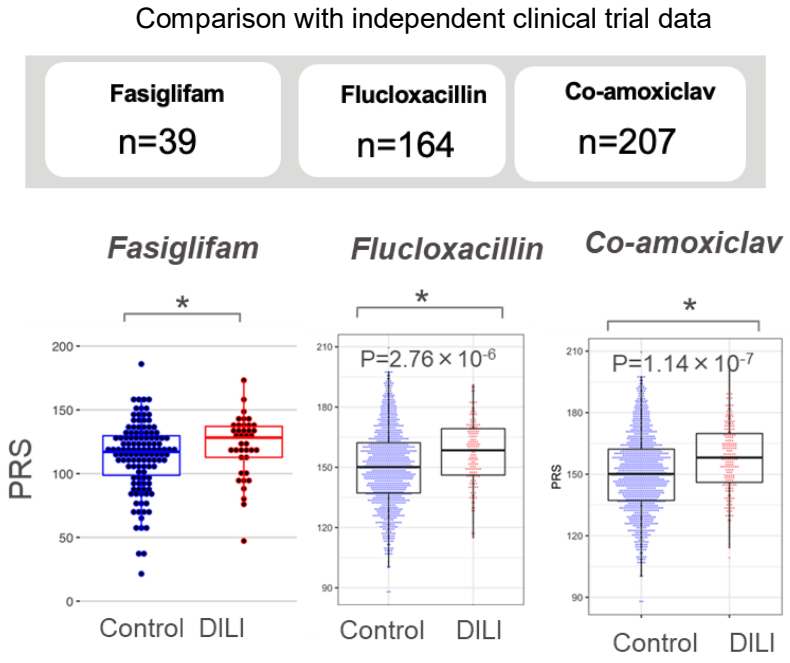


Figure2. Distribution of PRS in DILI patients and matched patients treated with drugs without DILI as controls. Fasiglifam (left), Flucloxacillin (middle), Co-amoxiclav (right).

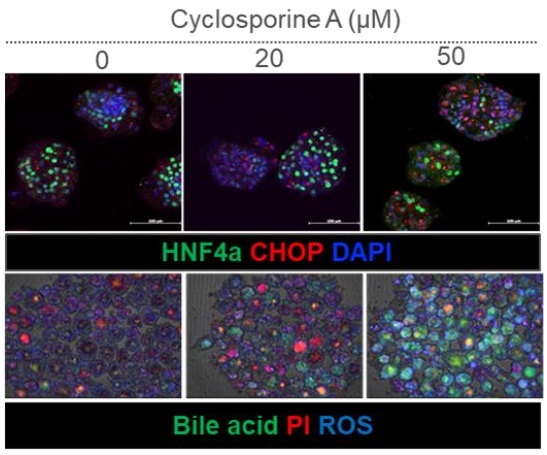
3. UPR and oxidative stress contribute to DILI susceptibility

Functional enrichment analysis of summary statistics for iDILIC/DILIN CM-DILI was revealed that pathways including mitochondrial activity, oxidant-induced survival, and ER stress are accumulated near the genetic polymorphism*8 that defines PRS (Figure 3A). Based on these pathway extractions, we further investigated CM-DILI vulnerability mechanisms using an iPSC-HLO model focusing on mitochondrial activity and ER stress signals under Cyclosporine A treatment (DILI drug). Consistently, we observed cholestatic cell death with elevated UPR-associated proteins, massive ROS production and functional mitochondria depletion in iPSC-HLO (Figure 3B). The enhanced antioxidant response induced by BM Bardoxolone Methyl (BM), a potent activator of Nuclear factor like-2 factor, protects against hepatocyte death in the PRS higher donor (Figure 3C). Collectively, the mechanism related to DILI susceptibility was elucidated for the first time, and the possibility of DILI prevention or therapeutic drug was also suggested.

A

Gene Set enriched in GWAS	P-value
PKA-mediated phosphorylation of CREB (Oxidative Stress)	6.43×10^{-3}
Activation of chaperone genes by XBP1s (ER stress)	9.86×10^{-3}
Citric acid cycle TCA cycle (Mitochondria activity)	1.04×10^{-2}

B



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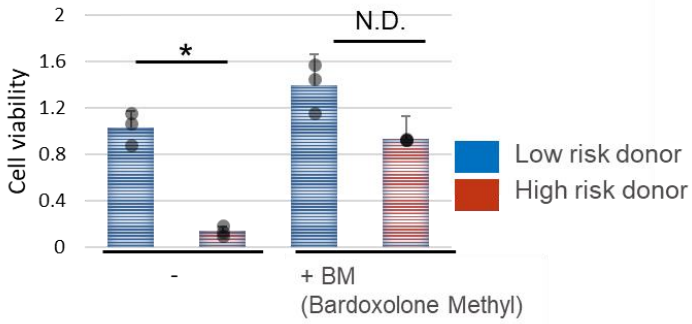

Figure3. Mechanistic association studies for DILI vulnerability.

A: Enriched experimentally known or related pathways associated with DILI from GWAS summary statistics in Nicoletti et al., 2017 were used.

B: Fluorescent imaging of human iPSC-derived liver organoid under Cyclosporine A treatment. Upper row shows endoplasmic reticulum stress marker CHOP elevation, lower row shows oxidative stress ROS increasing.

C: Cell viability upon BM treatment between PRS-high and -low donor-derived iPSC-HLOs under Cyclosporine A treatment.

【Words】

※1 PRS (Polygenic Risk Score)

an estimate of an individual's genetic responsibility to a trait or disease, calculated according to their genotype profile and relevant genome-wide association study (GWAS) ※7 data.

※2SNP (single-nucleotide polymorphism)

Difference of genomic DNA sequences between individuals, those with a high frequency (1%, etc.) in the population.

※3 DILI (drug-induced liver injury)

One of severe side effect caused by drugs, inflammation of the liver. It is classified into cholestasis/mixed type DILI and hepatocellular type DILI depending on the damaged cell types and the mechanisms.

※4 Organoid

A tissue structure that recapitulates an organ that exists in the living body.

This is an active field where many researchers have reported in recent years., and Takebe et al. have reported on the development of organoids of various organs in 2013, 2015, and 2017 Nature (Nature 499(7459):481-4, 2013), Cell Stem. Reported in Cell (Cell Stem Cell, 16(5):556-65, 2015) and Cell Reports (Cell Reports 21, 2661–2670, 2017).

※5 T-CiRA（Takeda-CiRA Joint Program for iPS Cell Applications）

Long term (10-years) joint research program between Kyoto University iPS Cell Research Institute (CiRA) and Takeda, established in 2015. Takeda provide a 20-billion-yen alliance fee. Under the direction of CiRA Director Shinya Yamanaka, the leader of researchers from CiRA, RIKEN, and Tokyo Medical and Dental University are leading the cutting-edge research toward clinical application of iPS cell technology.

※6 iDILIC

The International Serious Adverse Event Consortium (SAEC) is an international clinical network established in the UK for GWAS research. Abbreviation for International Drug-Induced Liver Network (iDILIC).

※7 DILIN

A consortium of academic institutions funded by the National Institutes of Health. Clinical information and genomic information of patients who have developed DILI due to alternative drugs such as over-the-counter drugs and supplements have been registered to be useful for DILI diagnosis and the like. Abbreviation for Drug-Induced Liver Injury Network (DILIN).

※8 GWAS (Genome-Wide Association Study)

A study that comprehensively searches for genetic polymorphisms*8 that affect individual differences. In GWAS for humans, tens of millions of genetic polymorphisms each provide statistics such as strength of association with traits such as disease onset. Abbreviation for Genome-Wide Association Study.

【Article information】

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